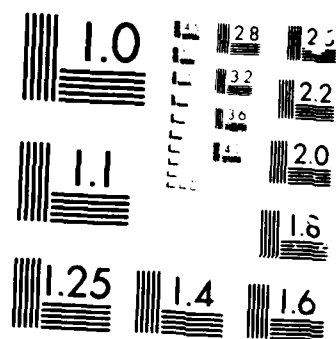


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ANNUAL REPORT

AUTONOMIC NERVOUS SYSTEM DISTRIBUTION AND FUNCTION IN LYMPHOID TISSUES ONR GRANT # N00014-85K0528 ,

I. General Scientific Goals

Specific Aims

The specific aims of this proposal are to extend our neuroanatomical and functional studies of the thymus gland to include an evaluation of the development, distribution and function of the ANS within specific tissues of the immune system. We propose to accomplish this by:

A. identifying the distribution of neuropeptides and transmitters within the interstitial nerves during the development of the mouse, rat, and chicken thymus, thymic-related lymph nodes, the bone marrow, the spleen, gut associated lymphoid tissue (GALT) and the chick bursa of Fabricius,

B. verifying when the need arises, the presence of these neurosubstances biochemically and characterizing any unique qualities they may express, and

C. evaluating both in vivo and in vitro the specific effects of these substances on thymic-related functions of the immune system.

II. General Scientific goals for the year June,1986 to June,1987

No changes are planned for the general scientific goals of this proposal.

III. Summary of the research June,1985 to June,1986

A. Overview

During the first year, the goals of our research were to continue the anatomical , biochemical and functional studies of the thymus gland as well as initiate studies on the innervation of other lymphoid tissues and organs. To date we have:

1. updated and extended our review of the development and distribution of autonomic nervous system innervation of the tissues and organs of the immune system,

2. completed a comparative study of the autonomic nervous system innervation of the mouse and the chick thymus,

3. revised and updated the developmental studies of the innervation of the normal thymus to include an analysis of ANS development and distribution within syngeneic embryonic thymuses transplanted beneath the kidney capsule of nude (athymic) mice,

4. identified via immunocytochemistry the presence of GABA-T (a putative marker for GABA and/or glutamate) within nerve terminals of the thymic cortex; initiated studies determining the presence of GABA, glutamate, and/or benzodiazapene receptors within the thymus and

5. biochemically characterized the nerve-related species of acetylcholinesterase within the thymus of normal and cortisone treated mice.

B. Experimental Results

Technical problems:

There have been no major technical problems encountered in the anatomical studies of the innervation of lymphoid tissues other than discovering that some of the commercial antibodies are not suitable for anatomical studies.

Some degree of difficulty was encountered with consistent collection of sucrose gradient fractions in our biochemical analysis of acetylcholinesterase. However, this problem has been eliminated by utilizing a Beckman Fraction Recovery System.

Research:

The research projects will be discussed in the order which they appeared in the Summary of the Research.

1. ANS Innervation of Lymphoid Tissues and Organs

Two reviews which updated an earlier description of the neuroanatomy of immune system tissues and organs (Neuroanatomy of the Immune System, K. Bulloch in Neuroimmune Modulation of Immunity 1985, Raven Press, eds. R. Guilleman et. al.) were written for separate symposia. Consequently, the emphasis of these two reviews are essentially different.

2. Comparative Study of ANS Innervation of Mouse and Chick Thymus

An anatomical study of the innervation of the chick thymus was carried out to determine if the source and terminal distribution of ANS innervation was comparable to that observed in the thymus of the mouse. Intrathymic injections of horseradish peroxidase (HRP) revealed that the CNS projections to the chick thymus are similar to but not identical to CNS projections to the mouse thymus. HRP - labeled neurons are present in the ventral horn of the chick's spinal cord in areas comparable to labeled neuron within the ventral horn of the mouse's spinal cord. A direct comparison of labeled neurons within the spinal cord of rodents and birds is difficult because of the difference in the anatomy of their spinal cords (15 cervical vertebrae for the chick versus 7 for the mouse) and of the location and anatomy of their thymuses.

In the chick's brainstem, HRP-labeled neurons are found in the nucleus of the ninth nerve and in the dorsal and ventral motor nucleus of the tenth nerve. In the mouse, HRP-labeled neurons are evident in the retrofacial nucleus of the ninth nerve and in neurons scattered throughout the nucleus ambiguus. However, in the mouse there are no labeled neurons observed in the dorsal motor nucleus of the tenth nerve.

The minor disparities between the chick and the mouse in the brainstem's projections to the thymus might be readily explained in light of the embryonic origin of the chick and mouse thymus. The avian thymus is derived from the third and fourth pharyngeal arch and pouch whereas the mouse thymus is derived solely from the third pharyngeal arch and pouch. During embryogenesis the third pharyngeal arch is innervated by neurons of the ninth nerve and the fourth pharyngeal arch is innervated by neurons of the tenth nerve. Since the chick thymus is derived from the third and fourth pharyngeal arch and pouch while the mouse thymus is derived solely from the third arch and pouch, the

presence of HRP-labeled neurons in the chick dorsal motor nucleus of the tenth nerve and the absence of these HRP-labeled neurons in this nucleus in the brainstem of the mouse is understandable. It is also possible that the preponderance of labeled neurons in the chick's dorsal motor nucleus of the tenth nerve might be explained by the fact that the ninth nucleus of the chick is not as well developed as it is in the mouse.

The presence of brainstem projections in the day-old chick indicates that the chick, like the mouse, receives vagal innervation during embryonic development (these neuronal centers represent the source for vagal efferents in the chick). The intrathymic distribution of these vagal cholinergic (AChE-positive) nerves in the chick thymus is similar to that found in the mouse's neonatal thymus in that the fibers are found within the medulla and at cortico-medullary boundaries. However, unlike the pattern in the neonatal mouse thymus, there is only a sparse AChE-positive innervation evident in the subcapsular area of the chick thymus. The AChE-positive nerves that innervate the subcapsular zone of the mouse thymus are derived from the phrenic and recurrent laryngeal nerves. Since the distribution of these nerves is confined primarily to the thoracic cavity, it is possible that in the chick they do not participate in the innervation of structures that lie in the cervical neck region.

The development of sympathetic catecholaminergic (CA) innervation of the thymus has already commenced in the day-old chick. These fibers are evident along the trabeculae, in perivascular plexuses at the cortico-medullary boundary and associated with the 5-hydroxytryptamine cells present in the cortex of the chick thymus. These findings are compatible with those studies which show that the CA fibers are evident in the mouse thymus during the third prenatal week.

The present study confirms the phylogenetic constancy of both the source and distribution of ANS innervation of the thymus gland. The fact that an anatomical network between the brain and the thymus is conserved testifies to the importance of this ANS - immune system relationship.

3. Development of ANS Innervation in Normal and Transplanted Thymuses

Our anatomical studies suggest that prenatal innervation of the thymus by the vagus nerve plays a significant role in the development and differentiation of the thymus gland. The results of these studies are summarized below.

During the early phases of thymic development, prior to the structural organization of the gland, acetylcholinesterase (AChE) - positive fibers (cholinergic) from the vagus nerve enter the primordium and are distributed to areas that define the future cortico-medullary boundary. After these vagal fibers are distributed within the thymus, the epithelial cells begin their rearrangement into the structural pattern seen in the adult thymus and the first t-lymphocyte precursors migrate into the gland. As the thymus descends from its cervical location, the thymic branch of the vagus nerve continues to develop complex nerve nets at the cortico-medullary boundary.

During the course of this caudal progression, other ANS parasympathetic and sympathetic nerves begin to penetrate the parenchyma of the thymus. The distribution of the phrenic and recurrent laryngeal nerves within the thymus occur after the penetration of the vagus and are mainly confined to the subcapsular regions. Sympathetic catecholaminergic (CA) innervation from the superior, stellate and other small ganglia of the sympathetic chain first appear within the murine

thymus at approximately embryonic day fifteen. The CA fibers are oriented primarily along the vasculature with perivascular plexuses common at the cortico-medullary boundary. Sparse free CA fibers not associated with blood vessels are evident within the medulla. In the cortex other CA fibers are found associated with a system of cortical autofluorescent (CAF) cells. Because of complex pathways of the sympathetic nervous system (nerves emanating from a given ganglia may travel in either a caudal or rostral path along the sympathetic chain before exiting with sympathetic nerves) we cannot determine by histochemical stains which ganglia contribute to which areas within the thymus.

The patterns of ANS innervation within the normal thymus were then compared to the development of innervation within viable embryonic, syngeneic thymus transplanted beneath the kidney capsule of the nude mouse. The results of these experiments show that the patterns of ANS innervation of the transplanted thymus are similar to that observed in the thymus of wildtype mice, occurring prior to the onset of thymic function.

Some transplanted embryonic thymuses did not evolve anatomically or functionally. Our analysis of these tissues revealed no detectable ANS innervation indicating; 1. a defect in the ability of the nude mouse thymus to sequester nerve fibers and 2. that innervation of the thymus during critical periods of development is an important component in the establishment of a functionally and structurally competent gland. These data corroborate the findings of those studies which show that specific recovery of function takes place after damage to the nervous system without reorganization of structure as long as the same type of nerves reinnervate the tissue [ie. taste buds will differentiate only in response to reinnervation by sensory nerves but not in response to reinnervation by motor nerves].

4. Identification of Neurosubstances Within Lymphoid Organs and Tissues

Our immunocytochemical studies indicates the presence of GABAergic-like nerves within the cortex of the thymus and in close association with the cortical autofluorescent cells. A less dense GABAergic innervation is evident within the spleen. These fibers are very fine and are distributed along the periarterial sheath and within the marginal zones of the lymphoid nodules. The density of GABAergic nerves within these two tissues correlates well with the biochemical analysis (J.G. Gerber and T.A. Hare, diabetes 28:1073,1979) which showed high levels of GABA within the thymus and low levels within the spleen. The effects of carbachol and GABA on thymocyte proliferation have been compared to that of Con A. Our preliminary data indicated that carbachol stimulates a separate population (or proliferation mechanism) of thymocytes whereas GABA and Con A stimulate the same population. The receptor binding assay clearly show the presence of benzodiazopene receptor within the thymus and possibly the presence of GABAergic receptors.

5. Thymic AChE Characterization in Normal and Cortisone Treated Mice

Low doses of cortisone have been reported to induce changes in the amount and distribution of AChE activity within areas of the thymus where thymocyte death had been independently noted under similar conditions (Muller & Muntener, Histochem. 60: 169, 1979). In order to further characterize this phenomena we have analysed the distribution and molecular species of acetylcholinesterase (AChE) within the murine

thymus gland utilizing biochemical and histochemical techniques.

Our earlier anatomical studies show that true AChE activity in the normal mouse thymus is confined to the dense network of the vagus nerve which terminates within the boundary between the cortex and medulla as well as to a subcapsular distribution of fibers derived from the phrenic and recurrent laryngeal nerves. Sucrose gradient analysis of the molecular species of AChE reveals that the low molecular weight, 4S globular molecule is the predominant form.

In the thymus of steroid-treated mice AChE activity increases over a 12 hour time period within the AChE-positive nerve-related areas and within areas that are associated with the GABA-T innervation of the cortex. By 36 hours the activity is found; in the thymocyte-depleted cortex of the gland, in the nerve terminals at the corticomedullary boundaries, and within eosinophils which have migrated into the thymus parenchyma. Several new molecular forms of AChE (10S & 16S) appear during the period of intense thymocyte death (t=10 hours) and remain throughout the test period. Cholinergic agents appear to modify the steroid-induced killing mechanisms of the thymocytes indicating that neuro-endocrine-immune interactions are part of the "normal" thymocyte selection process.

IV. Specific objectives for the coming year

Objective One: To further our understanding of the role that pre and postnatal innervation plays in the development and regulation of thymic function. Experiments will be initiated to study the effects of chemical and mechanical lesions of thymic nerves on the development and function of the thymus.

Objective Two: To determine if the GABA-T innervation of the thymus and spleen is representative of a GABA and/or glutamate innervation, and to analyze the presence of the appropriate neurotransmitter receptor for any or all of the above putative neurotransmitters.

Objective Three: To complete our studies on the steroid induction of nerve-related AChE within the thymus. Specifically we wish to know; if steroid induction of this enzyme within the thymus is age or sex dependent, if it is indicative of an efferent or afferent cholinergic response, and what role this enzyme might play (within the parasympathetic nervous system or as a putative hormone) in determining thymocyte functions.

Objective Four: To begin to extend our anatomical analysis of the distribution of putative neurotransmitters within other tissues and organs of the immune system.

List of Publications

Bullock, K., A comparative study of the autonomic nervous system innervation of the thymus in mouse and chicken. in Neuroimmune Modulation Gordon and Breach Edits. Spector et. al. in press.

Bullock, K. and Bossone, S. Nerve-related acetylcholinesterase (AChE) within the murine thymus: An indication of cholinergic modulation of thymic immune function. Annals of the New York Academy of Science submitted/in press

Bulloch, K., The Influence of the Autonomic Nervous System on the Development and Function of Immunity. Trends in Neurosciences, in press.
Bulloch, K. The Innervation of Immune System Tissues and Organs in Neuroimmunology eds. C. Cotman, B. Mc Ewen, A. Galaburda and D. M. Schneider, in press.

Bulloch, K., Cullen, M.R., Longo, D. and Schwartz, R.H., The development of autonomic nervous system innervation within normal and transplanted perinatal thymuses of the mouse. manuscript in prep.

Bulloch, K. A review of Neurohumoral Maintenance of Immune Homeostasis, E.A.Korneva, V.M. Klimenko and E.R. Shkhinek, Translated by S.A. Corson and E.O. Corson, Univ. of Chicago Press, 1985, in The Quarterly Review of Biology

Abstracts

Bulloch, K., and Cullen, M. R., A comparative study of the autonomic nervous system innervation of the thymus in mouse and chicken. Proceedings of the First International Workshop on Neuroimmunodulation. Ed. Spector et. al. 1986

Bulloch, K., Cullen, M.R., Robinson, S.R., Wu, J.-Y., and Cohen, S.R. Evidence for a GABAergic innervation of the murine thymus. Soc.Neurosci. Abstr., Vol. 11 Part 1, p.662, 1985.

Bulloch, K. The role of nerve-related AChE in the development and function of the thymus. Second International Workshop on NIM Yugoslavia June, 1986.

Bulloch, K. and McKeon, C., Cortisone induced changes in the nerve-related acetylcholinesterase (AChE) in the thymus Soc.Neurosci. Abst. 1986

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